# THE ANTIMICROBIAL ACTION OF HEPARIN[1]

JOHN F. CHRISTMAN[2] AND DAVID G. DOHERTY

*Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee*

Received for publication January 25, 1956

The earliest report on the bacteriostatic effect of heparin was made by Stoker (1949) who claimed that *Staphylococcus aureus* was inhibited by heparin only in the presence of protein (pus or trauma blood). Later Warren and Graham (1950) found, in contradistinction to the results of Stoker (1949), that *Bacterium stewartii* and *Micrococcus pyogenes* var. *aureus* were not inhibited by heparin when grown on a protein-containing medium (Difco brain heart infusion) but were inhibited when grown on a synthetic medium containing 0.01 per cent heparin. Growth of yeasts was not affected by the presence of heparin in the media. Additional contradictory evidence was obtained when a number of microorganisms, capable of utilizing heparin as a substrate for growth, were isolated by elective culture methods (Doherty and Christman, 1955; Christman and Doherty, 1956).

The present report represents an attempt to resolve the question of heparin inhibition and obtain a spectrum of inhibition of microorganisms by heparin. Four media of varying complexity were used, and a wide variety of microorganisms were tested for their ability to grow in these media with and without added heparin.

## MATERIALS AND METHODS

The organisms used were obtained from the American Type Culture Collection, the University of Tennessee Laboratories of Bacteriology,[3] and from Midwest Cultures. In addition, a number of organisms isolated by the elective culture method in which heparin was used as a substrate were tested. The composition of the four media is shown in table 1. These media were adjusted to pH 6.5, tubed in 4.0-ml quantities, and the tubes were divided into two groups. To one group, 1.0-ml quantities of heparin[4] solutions were added prior to sterilization, making the final

TABLE 1

*Composition of various media for testing the effect of heparin (all media were made up to 800 ml with water)*

| Additions | Medium I | Medium II | Medium III | Medium IV |
|---|---|---|---|---|
| Glucose | 5 g | 5 g | 5 g | 5 g |
| Ammonium sulfate | 4.5 g | 4.5 g | 4.5 g | — |
| $K_2HPO_4$ | 2.5 g | 2.5 g | 2.5 g | — |
| $KH_2PO_4$ | — | — | — | 5 g |
| Mineral salts* | 1 ml | 1 ml | 1 ml | — |
| Vitamin supplement† | 1 ml | 1 ml | — | — |
| Yeast extract | — | — | — | 10 g |
| Amino acid source | CH‡ | — | ASP§ | TRY¶ |

* Stock solution contained 50 mg of $MgCl_2$, 10 mg of $Na_2B_4O_7$, 10 mg of $FeCl_3$, 10 mg of $CaCl_2$, 1 mg of $MnCl_2$, and 10 μg of $Na_2MoO_4$ per ml.

† Contained 200 μg of thiamine hydrochloride, 100 μg of nicotinic acid, 100 μg of calcium pantothenate, 40 μg of pyridoxine hydrochloride, 0.5 μg of biotin, 500 μg of choline chloride, 1 mg of inositol, 5 μg of riboflavin, 10 μg of *p*-aminobenzoic acid, 10 μg of *p*-hydroxybenzoic acid, and 0.05 μg of vitamin $B_{12}$.

‡ CH = 5 g of vitamin-free, enzymically hydrolyzed casein.

§ ASP = 2 g of L-asparagine.

¶ TRY = 10 g of tryptone.

[1] Work performed under Contract No. W-7405-eng-26 for the U. S. Atomic Energy Commission.

[2] Research Participant in the Biology Division, Oak Ridge National Laboratory, under the auspices of the Oak Ridge Institute of Nuclear Studies 1954–55 on leave from the Department of Agricultural Chemistry and Biochemistry, Louisiana State University, Baton Rouge, Louisiana.

[3] The authors are indebted to Mr. D. O. Galloway for a number of these cultures.

[4] The heparin used was a purified material with an assay of 100 IU/mg supplied by Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE 2

*Heparin concentration and organism growth*

| Organism and Source or Strain | Maximum Percentage of Heparin in the Medium in Which Growth Will Occur | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | IA* | IF* | IIA | IIF | IIIA | IIIF | IVA | IVF |
| *Micrococcus citreus, Micrococcus pyogenes* var. *aureus* and *M. pyogenes* var. *albus* (Midwest) | 1 | 1 | NG† | NG | NG | NG | 1 | 1 |
| *Bacillus brevis* (Univ. of Tenn.) *Bacillus laterosporus* (Midwest) | 1 | 1 | 0.1 | 0.1 | NG | NG | 1 | 1 |
| *Bacillus subtilis* (Midwest) | 1 | 1 | 1 | 1 | NG | NG | 1 | 1 |
| *Bacillus cereus, Bacillus megaterium* (Univ. of Tenn.) | 1 | 1 | NG | NG | NG | NG | 1 | 1 |
| *Alkaligenes faecalis* (Univ. of Tenn.) | 1 | 1 | NG | NG | NG | NG | 1 | 1 |
| *Streptococcus pyogenes, Streptococcus faecalis* (Univ. of Tenn.) | 1 | 1 | NG | NG | NG | NG | 1 | 1 |
| *Proteus vulgaris* (Univ. of Tenn.) | 1 | 1 | 0.01‡ | 0.01‡ | 1 | 1 | 1 | 1 |
| *Proteus vulgaris* (Strain L)<br>*Paracolobactrum aerogenoides* (ATCC 11604; 61§, 51§, 54§)<br>*Paracolobactrum coliforme* (ATCC 11605)<br>*Paracolobactrum intermedium* (ATCC 11606)<br>*Escherichia coli* (ATCC 10795, 8739, 10586, Strain B)<br>*Bacterium cadaveris* (Gale strain)<br>*Aerobacter aerogenes* (Univ. of Tenn., 66§, 21§, 53§, 52§, ¶) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *Serratia marcesens* (Univ. of Tenn.) | 1 | 1 | 1‖ | 1‖ | 1 | 1 | 1 | 1 |
| *Salmonella enteriditis* (Univ. of Tenn.) | 1 | 1 | 1¶ | 1¶ | 1¶ | 1¶ | 1 | 1 |
| *Saccharomyces cerevisiae* (ATCC 4110, 4125, 4126, 9896), *Saccharomyces fragilis* (ATCC 8644), *Saccharomyces intermedius* (ATCC 2360), *Saccharomyces logos* (ATCC 10603), *Saccharomyces thermantitonum* (ATCC 563) | 1 | 1 | 1 | 1 | NG | NG | 1 | 1 |

* A, Medium in which the heparin is autoclaved. F, Medium in which the heparin is filter-sterilized and added aseptically.

† NG, No growth, when serially subcultured through 4 transfers.

‡ Growth on media containing 0.1 per cent heparin after serially subculturing on media of gradually increasing concentration of heparin.

§ Obtained through the elective culture method with heparin as substrate (Christman and Doherty 1956).

¶ Better growth in mediums IIA, IIF, IIIA and IIIF containing 1 per cent heparin in lower heparin concentrations or in absence of heparin.

‖ No pigment development in the presence or absence of heparin.

concentrations of heparin 1.0, 0.1, 0.01, 0.001, 0.0001, and 0.00001 per cent. The other group was sterilized, and filter-sterilized solutions of heparin were added aseptically. Thus, with each medium, one group contained autoclaved heparin and the other group contained filter-sterilized heparin.

The organisms were inoculated into each of the four media containing no added heparin and serially subcultured daily for four transfers. A number of the organisms failed to grow on media II and III, probably owing to the lack of amino acids essential for growth. The fourth transfer in the series was used as an inoculum for each medium containing heparin, and the tubes were inoculated in triplicate for each concentration of heparin. After 48 hr, the tube with growth in the highest concentration (usually 1 per cent), was

used as an inoculum for a serial subculture through three successive transfers. The organism was then transferred to the original heparin-free medium, allowed to grow, and then checked microscopically for any change or possible contamination. The results of these tests are presented in table 2.

## DISCUSSION

It is evident from the data in table 2 that, under the conditions of this test, most bacteria and yeast are not sensitive to antibiotic levels of heparin. Contrary to previous work (Warren and Graham, 1950), the composition of the media had no effect on the inhibition by heparin except in three organisms. In medium II, which was devoid of amino acids, the highest concentration of heparin which would permit growth of *Bacillus brevis* and *Bacillus laterosporus* was 0.1 per cent, whereas *Proteus vulgaris* grew in heparin concentrations only up to 0.01 per cent. The growth of all three was not inhibited by 1 per cent heparin in the more complete media—I, III, and IV. A possible explanation of this small inhibition may be that heparin interferes with the utilization of ammonia by these microorganisms. No difference was observed between the autoclaved and the filter-sterilized heparin media. Growth of *Aerobacter aerogenes* 52 and *Salmonella enteriditis* was better in the tubes containing 1 per cent heparin which possibly indicates that these organisms can rapidly metabolize heparin as a carbohydrate source.

## SUMMARY

Thirty-four microorganisms were tested for inhibition of growth by heparin in four media of varying complexity. No inhibition was observed in 31 cases at a 1 per cent level of heparin in the medium. In three cases, a small inhibition was obtained but only when the organism was growing on a medium lacking in amino acids.

## REFERENCES

CHRISTMAN, J. F. AND DOHERTY, D. G. 1956 Microbial utilization of heparin. J. Bacteriol., **72**, 429–432.

DOHERTY, D. G. AND CHRISTMAN, J. F. 1955 Microbial utilization of heparin. Bacteriol. Proc., **1955**, 141.

STOKER, S. B. 1949 Bacteriostatic action of heparin. J. Physiol., **110**, 26.

WARREN, J. R. AND GRAHAM, F. 1950 The effect of heparin on the growth of bacteria and yeasts. J. Bacteriol., **60**, 171–174.